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Reconstitution of human airway tissue in the humanized xenograft model

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Abstract

Normal human airway epithelial tissue may be reconstituted in the humanized xenograft model in immunodeficient NUDE mice. Epithelial cells dissociated from human fetal or adult tissue are seeded on a denuded rat trachea and implanted in the NUDE mice. After a first step of dedifferentiation, the human epithelial cells adhere on the denuded basal lamina of the rat host trachea and progressively reconstitute a normal well-differentiated epithelium after several steps of migration, proliferation, stratification and redifferentiation.

Keywords: Airway epithelium; Xenograft model; Differentiation; Epithelium reconstitution

1. Introduction

The response of airway surface epithelium to acute injury includes a succession of cellular events varying from loss of surface epithelium integrity to partial shedding of the epithelium or total denudation of the basement membrane. In order to analyze the molecular events involved in the airway epithelial regeneration after surface epithelial loss, an open tracheal xenograft model in NUDE mice has been developed [1]. Epithelial cells dissociated from human airway tissue and seeded on denuded rat trachea are implanted subcutaneously in the flanks of recipient NUDE mice. After dedifferentiation, the human epithelial cells adhere on the denuded basal lamina of the host trachea where they spread (step 1, see Fig. 1), migrate, proliferate, stratify (step 2) and then redifferentiate (steps 3 and 4). After 5 weeks, a normal pseudostratified well-differentiated respiratory epithelium is restored.

1.1. Origin of epithelial cells

Human airway surface epithelial tissue obtained from CF patients or non-CF individuals is collected from nasal polyps or bronchial resections. Tissues are soaked into transport medium and rapidly sent to the lab. After reception, tissues are (1) washed with Hank's HEPES; (2) incubated with Pronase E (1 mg/ml) overnight at 4 °C without agitation; (3) shook (30 s) in order to remove surface epithelium; and (4) centrifuged (10 min, 250 × *g*) to concentrate the cells. The pellet is resuspended in 1 ml of Hank's HEPES salts and completely dissociated by flushing the cells through a 0.5-mm-diameter needle. The airway epithelial cells are counted using a Malassez chamber. The trypan blue exclusion procedure is assessed using a 1:2 dilution in order to determine the number of living cells per milliliter. The airway epithelial cells are resuspended in GREEN medium for bronchial cells or in defined medium for nasal epithelial cells.

2. Inoculation of epithelial cells

After rehydratation of the assembly (rat trachea + tubing), the lumen is rinsed with washing buffer, and the sealing of the assembly is controlled. Epithelial cells are inoculated through a needle. After 15 min, the assembly is turned over to have homogeneity in cellular adherence to the denuded matrix.

3. Implantation in NUDE mice

At first, the mouse is anesthetized with intraperitoneal injection (pentobarbital 60 mg/kg). After 5–10 min, two incisions (length 0.5–1 cm) can be realized in the neck of the mouse. The assembly (tubing + trachea + epithelial cells) is implanted subcutaneously in the flanks of NUDE mice

Abbreviations: EGF, epidermal growth factor; DMEM, Dulbecco modified eagle's medium; SCID, severe combined immunodeficiency.

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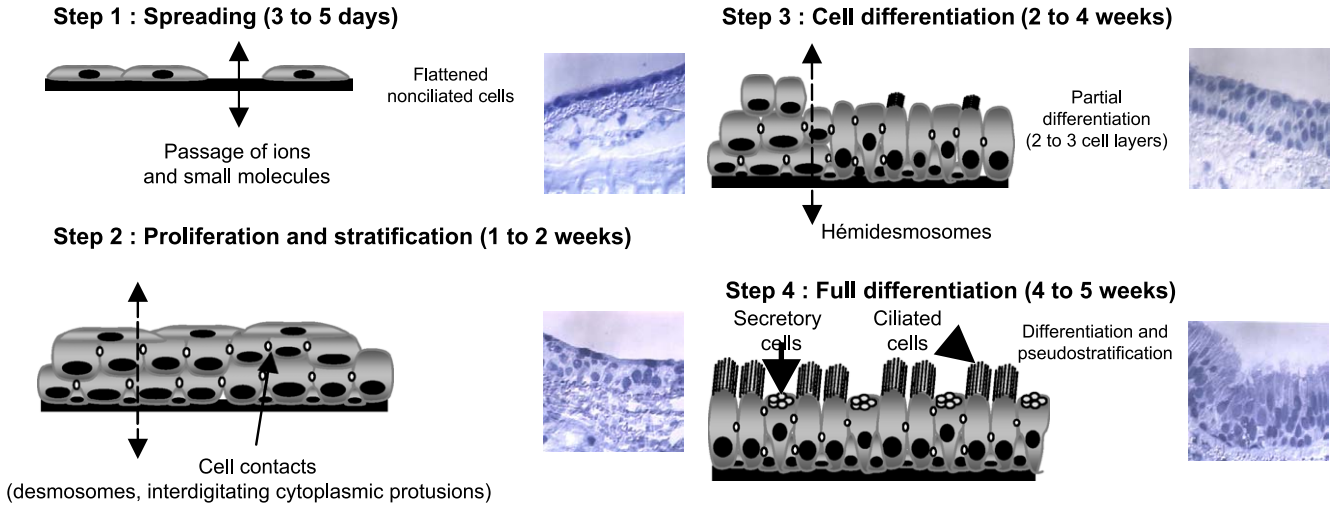

Fig. 1. Description of the four steps of human airway epithelium regeneration.

(two per mouse). The incision is closed using sutures. Tubings are closed using plastic caps.

The xenografts are rinsed twice a week with washing buffer. After 4–5 weeks, a pseudostratified epithelium is developed in the rat trachea (see Ref. [1]).

4. Materials

4.1. Culture media

Culture medium for human nasal epithelial cells (defined culture medium) were RPMI 1640; epidermal growth (EGF, 10 ng/ml); insulin (1 μg/ml); hydrocortisone (0.5 μg/ml); vitamin A (10 ng/ml); penicillin (200 U/ml); streptomycin (200 μg/ml); gentamicin (50 μg/ml); and amphotericin B (2.5 μg/ml).

Culture medium for human bronchial epithelial cells (GREEN medium) were Dulbecco modified eagle's medium (DMEM/F12, 3/4:1/4); insulin (5 μg/ml); hydrocortisone (0.5 μg/ml); cholera toxin (10 ng/ml); triiodothyronin (1.5 ng/ml); transferrin (6 μg/ml); EGF (10 ng/ml); penicillin/streptomycin (100 U/ml, 100 μg/ml); gentamicin 50 μg/ml; and adenine (20 μg/ml).

Washing buffer were Hank's HEPES salts; penicillin (600 U/ml); streptomycin (600 μg/ml); gentamicin (150 μg/ml); and amphotericin B (7.5 μg/ml).

4.2. Others

Animals: 45- to 47-day-old male rats (Wistar), weight in the range of 220–250 g; NUDE mice (*nu*/*nu*), 8 weeks, female (Charles River or IFA CREDO, France).

Tubing: elastic tubing (Silastic), ∅ inside 0.76 mm, outside 1.65 mm (Lambert Rivière SA, France); polyethylene tubing, ∅ inside 1.14 mm, outside 1.57 mm (Etablissement Aubry, France); threads: sutures (Harvard Biosciences, France); anaesthesia: pronase (60 mg/kg); Pronase E Fibronectin-binding proteins of Staphylococcus aureus are involved in adherence to human airway epithelium (1 mg/ml; Sigma).

5. Procedure

5.1. Tubing sterilization

The tubing is sterilized in alcohol (70°) during 24 h. At this step, all experimental steps are sterile.

5.2. Tracheal rat treatment

The tracheas are frozen and thawed (two cycles) to remove the rat surface tracheal epithelium. Tracheae of adult Wistar rats are dissected properly, and the lumen of rat tracheae are rinsed with washing buffer in order to remove all cell debris. The tracheae are frozen (first freezing) at − 80 °C. Tracheas are thawed (first thawing) and are tied at their ends to sterile tubings (see Fig. 2), the sealing of the assembly is then controlled (no escape of washing buffer), and the assembly is stored at − 80 °C (second freezing). The assembly is thawed (second thawing), rehydrated in washing buffer and rinsed before inoculation of the dissociated epithelial cells (1×10^6 cells per trachea in 80 μl culture medium containing 10% fetal calf serum).

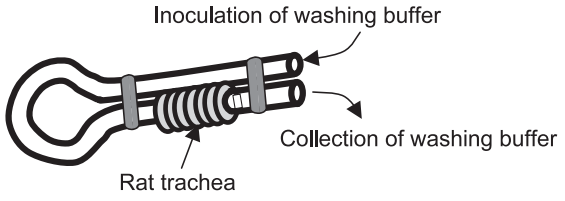

Fig. 2. Scheme of a tracheal assembly. Tracheae tied at their ends to sterile tubings are shown in the figure.

6. Conclusion

This humanized chimeric model has the main advantage of reconstituting a human adult airway epithelium exposed to the air environment as in adult human airways.

Several applications have been described including the analysis of airway epithelium regeneration, the evaluation of drug and vector efficiency, the study of host–pathogen interactions and the analysis of the airway surface liquid secreted by the reconstituted human airway epithelium [2–6].

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